International Research Journal of Environment Sciences ISSN 2319–1414
Vol. **4(4),** 44-53, April **(2015)** *Int. Res. J. Environment Sci.*

Biodegradation of Dye Reactive Black-5 by a Novel Bacterial Endophyte

Sharma Shilpa and Roy Shikha
Department of Botany, University of Rajasthan, Jaipur, INDIA

Available online at: www.isca.in, www.isca.me
Received 31st January 2014, revised 5th March 2015, accepted 6th April 2015

Abstract

The dye Reactive Black-5 was observed to be decolorized and degraded efficiently by a novel bacterial endophyte isolated from plant Amaranthus spinosus, collected from the site contaminated with effluents from textile dyeing and printing industries in Sanganer, Jaipur, Rajasthan. The16SrDNA analysis identified the bacterial endophyte to be Exiguobacterium profundum strain N4. The Exiguobacterium profundum strain N4 efficiently decolorized 901ppm of dye upto 84.78 % after 12 hrs of incubation under static condition at 34^{0}C. Beef extract upto 2g/ltr. and glucose upto 10 ml/ltr were found to be most favorable for attaining maximum biodecolorization with the isolated bacterial endophyte. UV–Vis spectroscopy, HPLC and GC-MS analysis confirmed biodegradation of the azo dye. A probable biodegradation pathway was proposed with the help of GC-MS analysis. Phytotoxicity studies with seeds of the plants Triticum aestivum and Phaseolus mungo, confirmed non-toxic nature of the extracted metabolites, while the dye exhibited toxicity.

Keywords: *Exiguobacterium profundum strain N4*; *Amaranthus spinosus*; Reactive Black-5; *Endophyte.*

Introduction

Colored textiles have always fascinated mankind. Textiles are colored by the use of dyes during the process of dyeing and printing. Textile dying and printing is among the oldest industrial sectors. Earlier dyeing and printing was carried using natural dyes, but with the advent of industrial revolution synthetic dyes have come in demand due to their vast range of colors, lower costs and their easy process of production, therefore, synthetic dyes have almost replaced natural dyes. Synthetic dyes are aromatic organic compounds. Azo dyes constitute the largest class of synthetic dyes. Due to their low fixation rate these dyes are released along with the effluents from textile dyeing and printing industries. The presence of dyes in the effluent makes them colored. These untreated effluents are released into nearby water bodies as well as soil, posing a serious threat to the aquatic life and to the health of human beings living in and around the area.

There are various physiochemical methods available for the removal of dyes from waste water but a number of drawbacks are associated with each of them, therefore, there is a need for a method which is efficient and also meets the environmental regulatory requirements. The use of microorganisms has been emphasized from past few years for the removal of toxic azo dyes. Therefore an attempt has been made in the present study to isolate endophyte which is efficient in biodecolorizing and biodegradaing these textile azo dyes. As it has already been reported that bacteria degrading recalcitrant compounds are more abundant among endophytic populations than in the rhizosphere of plants in contaminated sites[1,2], implying that endophytes have a role in metabolizing these substances. Earlier endophytes with potential for bioremediation has been studied, *Methylobacterium populi BJ001* endophytic bacterium isolated from hybrid *poplar* trees was capable of degrading the explosives TNT, RDX and HMX[3,4] and *Pseudomonas putida VM1450* isolated from *Pisum sativum* was capable of degrading 2,4-D[5]. The efficient bacterial endophyte was identified through 16SrDNA. The pathways of the selected azo dye biodegradation have also been proposed and the phytotoxicity of the extracted metabolites has also been investigated.

Material and Methods

Chemicals and azo dye: The dye Reactive Black- 5 (diazo) was a vinyl sulfone based reactive azo dye that was manufactured by Metro chem. Industries Ltd. Ahmedabad and was procured from textile dyeing and printing unit at Sanganer. The dye was chosen due to its wide applicability in textile dyeing and printing industries. The dye was used without carrying any further purification. The chemicals used were of analytical grade.

Isolation of Bacterial Endophytes: The bacterial endophytes were isolated from the roots of the healthy flowering plant *Amaranthus spinosus* collected from a unique area which was contaminated with untreated textile dyeing and printing effluents and was processed immediately after collection. The isolation was done according to the following procedure: plant samples were washed under running tap water for 10-15 mins. Air-dried and roots were separated out. The plant roots were weighed one gram on a weighing balance. The samples were then surface-sterilized by dipping in 70% ethanol for 1 minute, with 2% sodium hypochlorite for 10 minutes and then treated with 70% ethanol for 30 sec. followed by rinsing five times in sterilized distilled water. The surface sterilized samples were blot-dried using sterile filter paper. The samples were then

macerated in one ml of distilled water. For the macerated root sample serial dilutions were made upto 10^{-5} dilutions. One hundred micro liters from each dilution of the respective sample was then poured in their respective petri plates so labeled from 10^{-1} to 10^{-5} containing nutrient agar medium. The plating was done in triplicate for each dilution. The plates were incubated at 37^0 C for 72 - 96 hours. Sterility check was performed by imprinting the surface sterilized plant root samples in the media. The isolated bacterial endophytes were maintained as pure cultures on nutrient agar media.

Screening for dye biodecolorization: All biodecolorization experiments were conducted in MB agar medium containing (g/L): Na_2HPO_4-6, KH_2PO_4-3, NH_4Cl-1, NaCl-0.5, D-Glucose- 10ml, beef extract- 2, $MgSO_4.7H_2O$- 1ml, Thiamine - HCl solution – 1ml, $CaCl_2$- 1ml, d H_2O – 1L, pH-7. The composition of trace elements solution was (g/L): $CoCl_2.6H_2O$-0.2 g/l, H_3BO_3-0.3 g/l, $ZnSO_4.7H_2O$-0.1g/l, $MnCl_2.4H_2O$-0.03 g/l, $Na_2MoO_4.H_2O$-0.03 g/l, $NiCl_2.6H_2O$-0.02 g/l, $CuCl_2.2H_2O$-0.01g/l, pH-3.4. The agar plates contained 20g/l of agar.

Initially competent bacterial endophytes were screened by inoculating separately each of the purified bacterial endophtyte on Mineral Base agar plates containing 150 ppm of dye. The plates were incubated at 37^0 C for 72 - 96 hours. Uninoculated plates with the dye were used as control. The experiment was conducted in triplicate. Plates were regularly observed for the zone of clearance around the bacterial colony for a period of 4 days. The competent bacterial endophytes with maximum zone of clearance were selected.

The efficiency of the competent endophytes was tested in MBM broth. Each competent bacterial endophyte was inoculated separately in flasks containing MBM with 150 ppm of the dye and incubated at 37^0 C for 72 - 96 hours under static conditions. Flasks containing MBM with the dye but no bacterial inoculum were used as control. The experiment was conducted in triplicate. % Decolorization was measured separately on UV-Visible spectrophotometer for each of competent endophyte using supernatant obtained after centrifugation of 5 ml sample at 10,000 rpm for 15 mins at 4^0C. Dye decolorization was measured by monitoring the absorbance at maximum absorption wavelength 598 nm for dye Reactive Black-5 using a UV-Vis spectrophotometer (UV-1700 Pharmaspec Shimadzu). The percentage of decolorization was calculated according to the following formula:

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance value-final absorbance value}}{\text{Initial absorbance value}} \times 100$$

The efficient bacterial endophytes with maximum % decolorization were then selected.

Optimization studies: A loopfull of efficient bacterial endophytes was inoculated in the MB media under static and shaking conditions. The effect of various carbon sources like Glucose, Galactose, Lactose and Maltose and the nitrogen sources viz., beef extract, yeast extract, malt extract and peptone were also evaluated. Similarly the effect of different dye concentrations at 150, 300, 600 and 901ppm of the dye as well as the effect of different incubation temperatures was also evaluated at 30, 32, 34 and 36^0C. Aliquots (5ml) of the media were withdrawn after 12 hrs of incubation and centrifuged at 10,000 rpm for 15min at 4^0C to separate the supernatant. % Decolorization of the dye was analyzed at 598 nm using UV–Vis spectrophotometer (UV-1700 Pharmaspec Shimadzu).

Characterization and identification of efficient bacterial endophyte: The preliminary morphological and biochemical characterization of the most efficient bacterial endophyte was carried out. The morphological traits that were analyzed during the present study includes: colony type, margin of the colony, its elevation, color of colony, its surface, the opacity of colony whereas the biochemical traits included: Gram's reaction, capsular staining, carbohydrate fermentation test, indole test, citrate test, catalase test, MR-VP, hydrogen sulphide production test, gelatin test, starch and motility test. The identification was done using 16SrDNA carried out by Xcelris Labs Ltd. Ahmedabad. DNA was isolated from the culture. Its quality was evaluated on 1.2% Agarose Gel, a single band of high-molecular weight DNA was observed. Fragment of 16SrDNA gene was amplified by PCR from the above isolated DNA. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose Gel. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 27F and 1492R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 1416bp 16SrDNA gene was generated from forward and reverse sequence data using aligner software. The 16SrDNA gene sequence was used to carry out BLAST with the nrdatabase of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 5 [6]. The evolutionary history was inferred using the Neighbor-Joining method[7].

Analytical studies: The biodecolorization of the dye Reactive Black-5 was monitored by using UV–Vis spectrophotometer (UV-1700 Pharmaspec Shimadzu) whereas biodegradation was evaluated by using HPLC and GC-MS. Following 12 hrs of incubation with the efficient bacterial endophytes AMR-1 and AMR-7, the decolorized culture media was centrifuged at 10,000 rpm for 15 min at 4^0C and supernatant obtained was then used for determining the possible changes in the absorption spectra of the azo dye in U. V. - Visible range from 250-700 nm. The metabolites were extracted thrice with equal volume of ethyl acetate. The extracts were then dried over anhydrous Na_2SO_4 and evaporated to dryness. The biodegradation analysis was done by dissolving the recovered metabolites in methanol. HPLC analysis was carried out using Hewlett Packard Series-

1100. The mobile phase was methanol: water (9:1, v/v) with flow rate of 0.75 ml/min. The identification of metabolites formed after degradation was carried using GC-MS Varian 4000 containing a Hewlett Packard MS Engine, equipped with integrated gas chromatograph with CP 8944 column (30 m long, 0.25 mm, 0.39 mm film, thickness 0.2 µm). Helium was used as a carrier gas at a flow rate of 1 ml min^{-1}. The ionization potential was 70 eV. The injector temperature was maintained at 280^0C; column temperature starting at 70^0C for 5 min increased @ 15^0C min^{-1} up to 280^0C for 10 min. The extracted metabolites were identified by comparison of retention time and mass spectra with fragmentation pattern, with the help of GC-MS.

Phytotoxicity study: In order to assess the toxicity of the dye Reactive Black-5 phytotoxicity study was performed. The study was carried out using 10 seeds each of plants *Triticum aestivum* (monocot) and *Phaseolus mungo* (dicot) by adding separately 25 ml sample of the Reactive Black- 5 (901 ppm) and its degradation products and incubating at room temperature. In case of control set distilled water was used. Germination (%) and length of plumule and radical were recorded after 7 days. The experiment was carried out in triplicate.

Results and Discussion

Isolation of Bacterial Endophytes: A total number of sixteen bacterial endophytes were isolated from the roots of the plant *Amaranthus spinosus*. These bacterial endophytes were named according to the plant source and the plant part from which they were isolated and followed by a number (AMR1 to AMR16 with AM = Amaranthus and R= Root). As per the author's knowledge this is the first report of bacterial endophytes being isolated from plant *Amaranthus spinosus*, although, endophytic bacteria have earlier been isolated from different plants but not from *Amaranthus spinosus*[8,9].

Screening for dye decolorization: Bacterial endophytes isolated from the plant *Amaranthus spinosus* were screened for their dye decolorizing ability on solid MB media. Most of bacterial endophytes were competent of decolorizing the dye that is out of 16 bacterial endophytes 12 were able to decolorize the dye. The bacterial endophytes AMR-1 and AMR-7 had the maximum average zone of clearance among all the other competent isolates.

The competent bacterial endophytes showing maximum zone of clearance AMR-1, 2, 3, 5, 7, 8 were then further tested for their efficiency of dye decolorization in mineral base broth medium. The percent decolorization of bacterial endophyte AMR-1 was 81.64% whereas that of AMR-7 was 77.80% figure-1, which was maximum among the competent bacterial endophytes. Therefore the bacterial endophytes AMR- 1 and AMR-7 were selected for optimizing the culture conditions so as to achieve maximum biodecolorization of the dye Reactive Black-5.

Optimization studies: Effect of static and shaking conditions: The bacterial endophytes showed better percent decolorization under static conditions as compared to those under shaking conditions. The percent decolorization under static condition of bacterial endophyte AMR-1was 83.31% while under shaking condition it was 23.00%, whereas that of AMR-7 under static condition was 80.80 % and that in shaking condition was 31.14 % in 12 hrs with the dye concentration at 150 ppm. It was observed that along with the decolorization process there was no change in pH as well as there was no adsorption of color onto the surface of bacterial endophytes. These observations confirmed that biodecolorization of the dye was due to biodegradation rather than adsorption. It was also emphasized that static conditions were necessary for the biodecolorization. Therefore, further optimization studies were carried out under static conditions only.

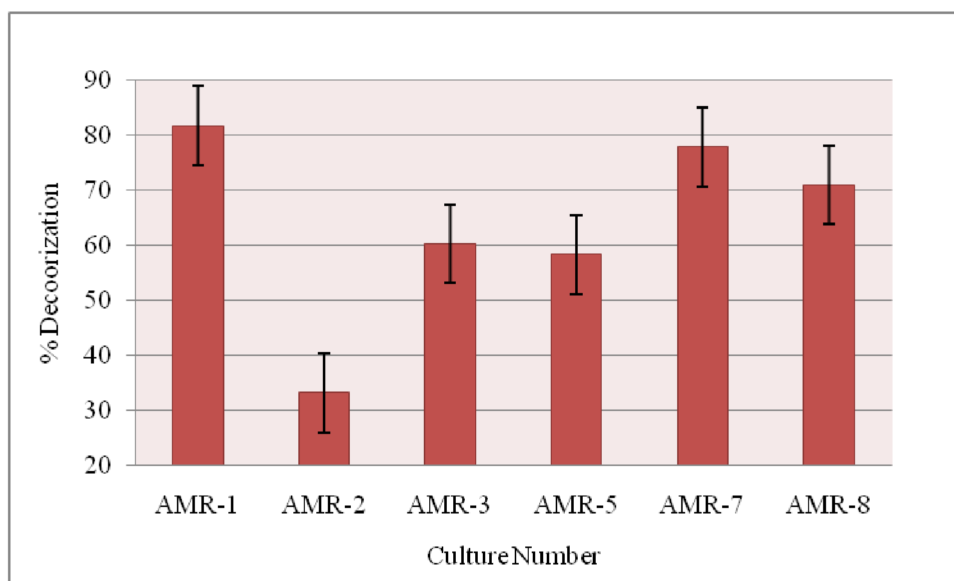

Figure-1
Competent bacterial endophytes along with their calculated % decolorization

The static condition was favorable for biodecolorization, which could be due to the involvement of azo reductases enzyme for the biodecolorization of azo dyes, which has also been reported by Pandey et al. [10] and others. Biodecolorization by microorganisms may take place in two ways: either by adsorption (or biosorption) on the microbial biomass or biodegradation by microbes[11]. It was observed that along with the biodecolorization process there was no change in pH as well as there was no adsorption of color onto the surface of bacterial endophytes. These observations confirmed that biodecolorization of the dye was due to biodegradation rather than adsorption.

Effect of carbon sources: It was observed that percent decolorization of the dye was maximum when 10ml/ltr of Glucose was used as the source of carbon. Although the acclimatized bacterial endophytes AMR-1 and AMR-7 were able to use dye as the sole carbon source, as also been reported by Bheemaraddi et.al. [12], but the percent decolorization was very low as compared to when the carbon source was supplied from outside. There appears to be some doubt on the effect of glucose on biodecolorization and biodegradation of azo dyes by bacterial strains as some scientists[13] reported positive effect of glucose on the biodecolorization while other group of scientists [14, 15] had opposite opinion.

Effect of nitrogen sources: The percent decolorization *was greatly enhanced* when beef extract was used as nitrogen source. Moreover 2 gms/ltr of beef extract was found to be most favorable to achieve maximum biodecolorization with bacterial endophytes AMR-1 and AMR-7, similar finding has also been reported by Saratale et.al. [14]. It was observed that the nitrogen source was absolute necessity to achieve higher percent decolorization with the efficient strains. It was also observed that both glucose as well as beef extract were readily assimilated by AMR-1 and AMR-7 that helped in achieving growth as well as maximum biodecolorization. This has also been reported earlier on other dyes[16,17].

Effect of dye concentration: The percent decolorization of Reactive Black-5 was affected with dye concentration from 150 ppm to 901 ppm. The percent decolorization of bacterial endophyte AMR-1 was 85.98% whereas that of AMR-7 was 82.29% with 150 ppm concentration of dye Reactive Black -5 whereas with 901 ppm concentration of dye percent decolorization of AMR-1 was 84.78 %, whereas that of AMR-7 was 81.45 %. It could be inferred that the increasing concentration of dye affected percent decolorization as also been reported by Moosvi S. and Kalyani D.C. et. al.[17,18]. However there was no adverse effect of dye concentration on the growth of AMR-1 and AMR-7 at least upto 901ppm of dye concentration.

Effect of temperature: The bacterial endophytes AMR-1 and AMR-7 showed better percent decolorization at 34^0C temperature. Although, the endophytes were found competent to grow on a wider temperature range but 34^0C was found to be most favorable to achieve maximum biodecolorization. The temperature variation below and above this temperature was not much favorable for biodecolorization.

Characterization and identification of efficient bacterial endophyte: The preliminary study was on morphological characteristics of the bacterial endophytes AMR-1, isolated from roots of the plant *Amaranthus spinosus.* The characteristics observed were: Gram-positive, rod-shaped, motile and circular with entire margins. The biochemical characteristics examined are depicted in table-1. The nucleotide homology of this strain showed that it was most similar to the genus *Exiguobacterium*. Further phylogenetic analysis figure-2 identified the endophyte as *Exiguobacterium profundum strain N4* (Gen Bank Accession Number: KF928335.1). Earlier there have been reports on the isolation of the same genus from variety of different habitats [19, 20]. However there is no report available regarding the decolorization and degradation of azo dye Reactive Black-5 using the strain *Exiguobacterium profundum strain N4.*

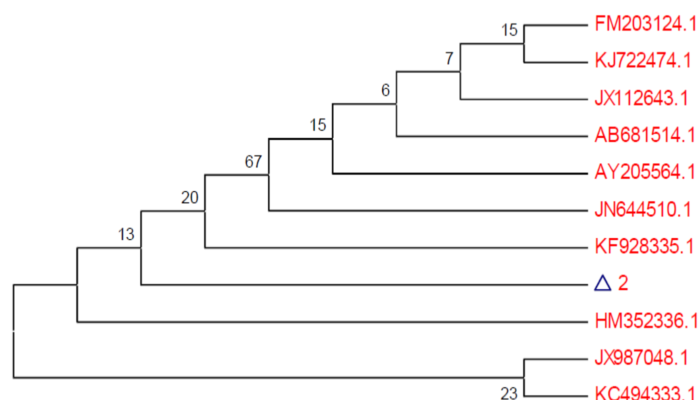

Figure-2
The evolutionary history was inferred using the neighbor-joining method

Table-1
The biochemical characteristics of the bacterial endophyte AMR-1

Culture No.	Gram's reaction		Sugar Fermentation					Indole	Citrate	Catalase	MR	VP	Gelatin	Starch	Motility	Capsular Staining
	R_X	Shape	Dextrose	Sucrose	Lactose	Gas	H_2S									
AMR 1	G +	Rod	+	-	+	No	No	-	+	++	-	+	+	+	+	-

Analytical studies: The results of U.V. - Visible spectrophotometric scanning (250-700 nm) showed complete biodecolorization under static conditions. In control the peaks were clearly visible (blue color line) Figure-3 whereas in sample they had disappeared completely (red color line). The analyses also showed a sharp decrease in absorbance in the visible range. Disappearance of peaks both in the U.V. range as well as in the visible range clearly indicated complete degradation. Moreover AMR-1did not retain any color on the cell wall indicating that there was no adsorption mechanism involved and the color removal was the result of biodegradation. The difference with both the cultures AMR-1 and AMR-7 was that with AMR-1 there was a sharp decline in the absorbance values unlike AMR-7. Furthermore, the extent of biodegradation was much greater with AMR-1 as compared to AMR-7. Maximum biodecolorization obtained under the present study with *Exiguobacterium profundum strain N4* was 84.78 % after incubation for 12hrs. at 901ppm of the dye under optimum environmental condition. Earlier Wang X et al.[21] reported biodecolorization of reactive black-5 using *Rhodopseudomonas palustris* and Hussain et al.[22] using *Pseudomonas* sp.RA20 whereas Dhanve et al.[16] had also reported biodecolorization up to 91.2% within 48 h at static condition using *Exiguobacterium sp.* of the dye reactive dye Navy blue HE2R, Tan et al.[23] had also reported decolorization with Exiguobacterium sp. for azo dyes.

Biodegradation of the dye Reactive black-5 was further confirmed by HPLC analysis of the control and the extracted metabolites obtained after biodecolorization. The HPLC profile of the control medium figure-4a showed major peaks at retention time 0.763, 0.860, 1.548, 1.684, 1.831, 2.076, 2.381and 2.668 min, while the extracted metabolites from biodecolorized medium with the AMR- 1 showed peaks at 0.806, 1.183, 1.549, 1.684, 2.007, 2.319, 3.286 and 3.745 min, having different retention times than that of the control medium figure-4b whereas those inoculated with AMR-7 showed peaks at retention times 0.823, 1.234, 1.545, 1.682, 1.827, 2.074, 2.348, 2.651, 3.229 and 3.742 min. No peak corresponding to retention time to that of the parent dye compound was observed. Absence of peaks corresponding to the retention time to that of the parent dye indicated complete biotransformation of the dye to other simpler products and confirmed biodegradation of the dye. When the dye reactive black-5 was biodegraded by AMR-1, the metabolites formed were less aromatic and more polar compounds, since the metabolites peak area decreased and shifted towards lower retention time.

GC-MS analysis revealed the presence of several peaks. The dye Reactive Black-5 with the molecular weight 991.82 upon degradation by the endophytic bacterial cultures AMR-1 figure-5 and AMR-7 revealed metabolites with much lower molecular weights. The mass spectra of the AMR-1 showed biodegradation of the selected dye to benzene and naphthalene which are non-toxic.

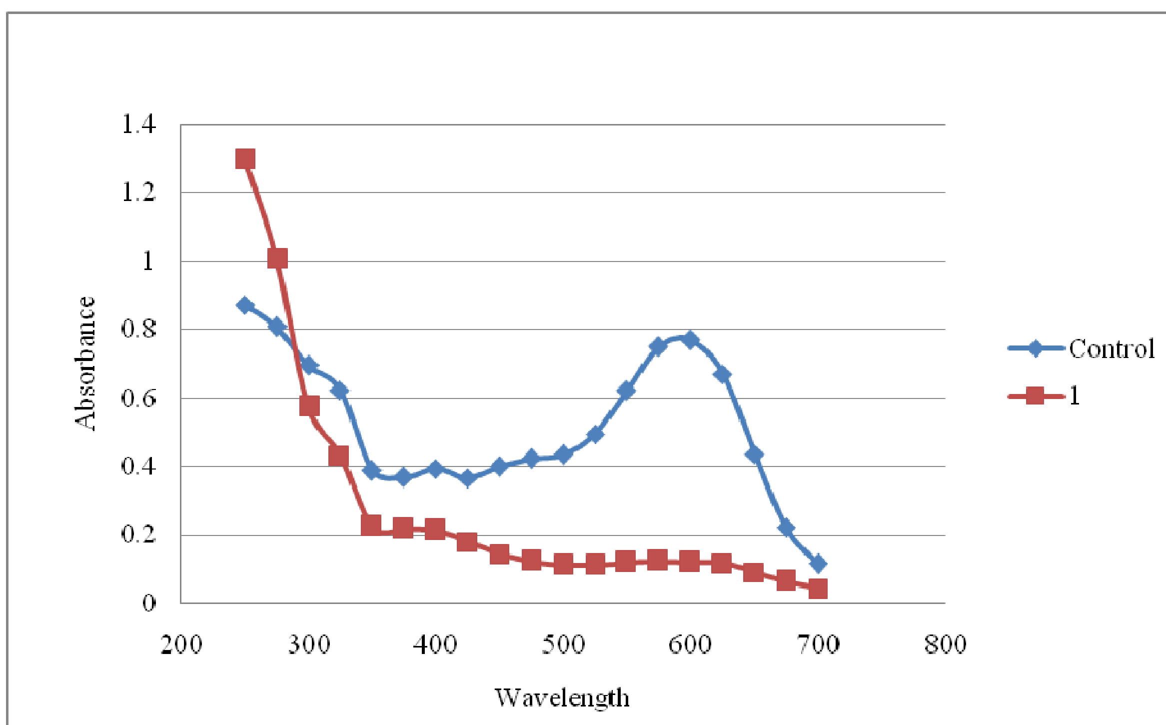

Figure-3
Biodegradation pattern of Reactive Black-5 for AMR-1

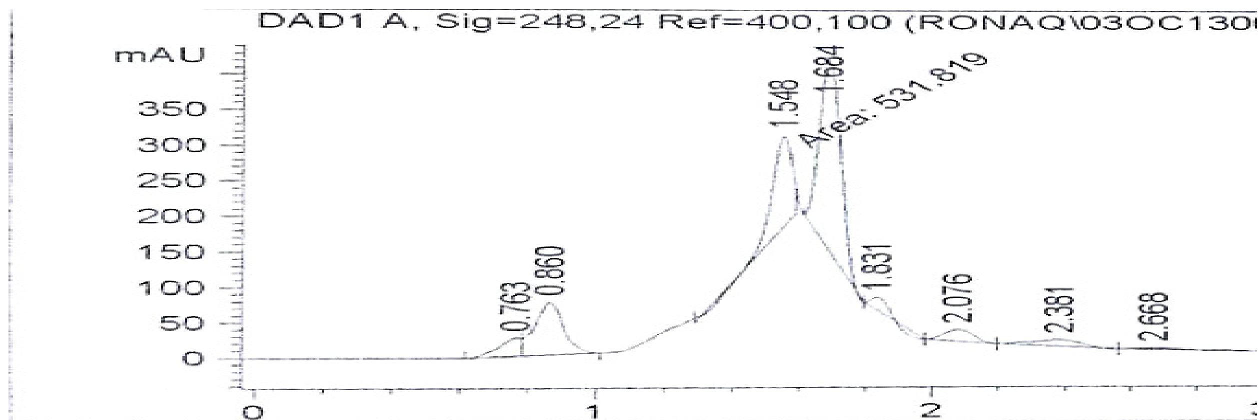

Figure- 4a
Peaks for control dye Reactive Black-5

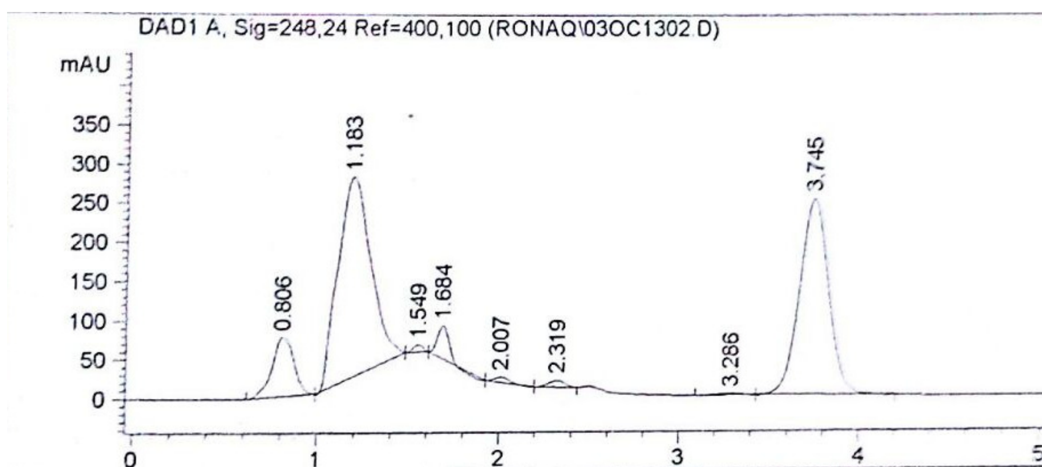

Figure-4b
HPLC chromatogram of the extracted metabolites

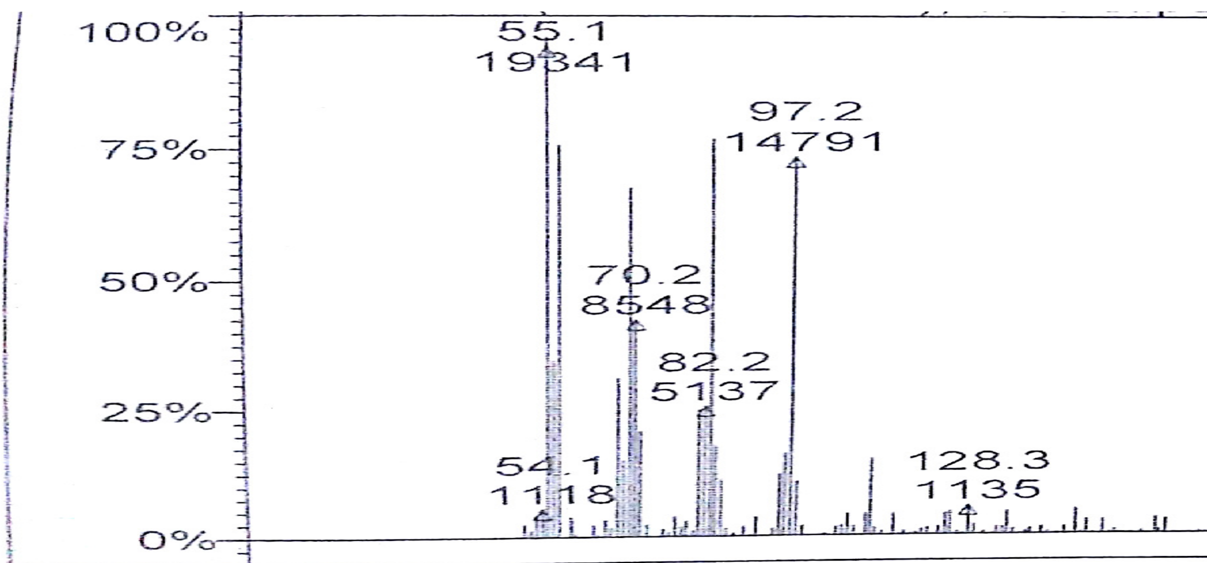

Figure-5a
GC-MS mass spectra of AMR-1

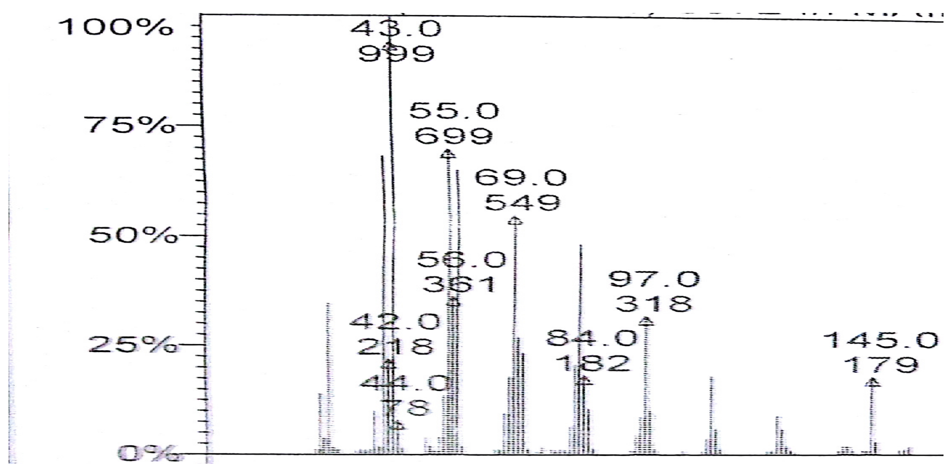

Figure-5b
GC-MS mass spectra of AMR-1

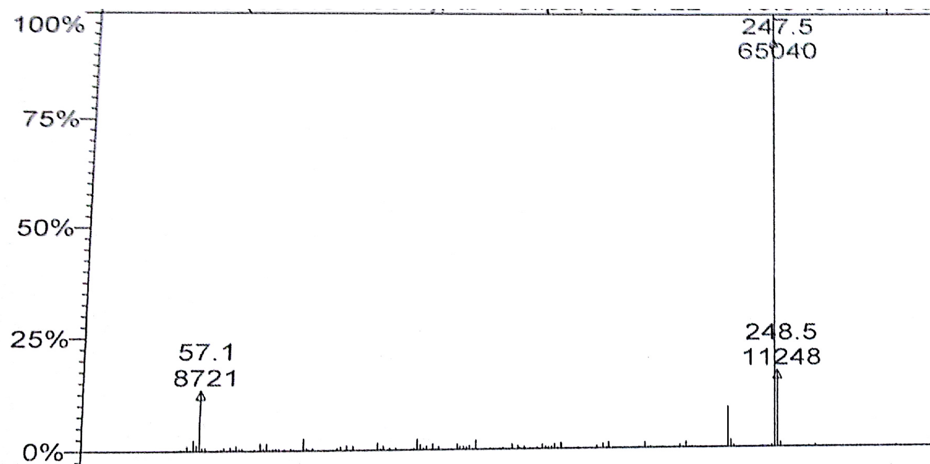

Figure-5c
GC-MS mass spectrum of AMR-7

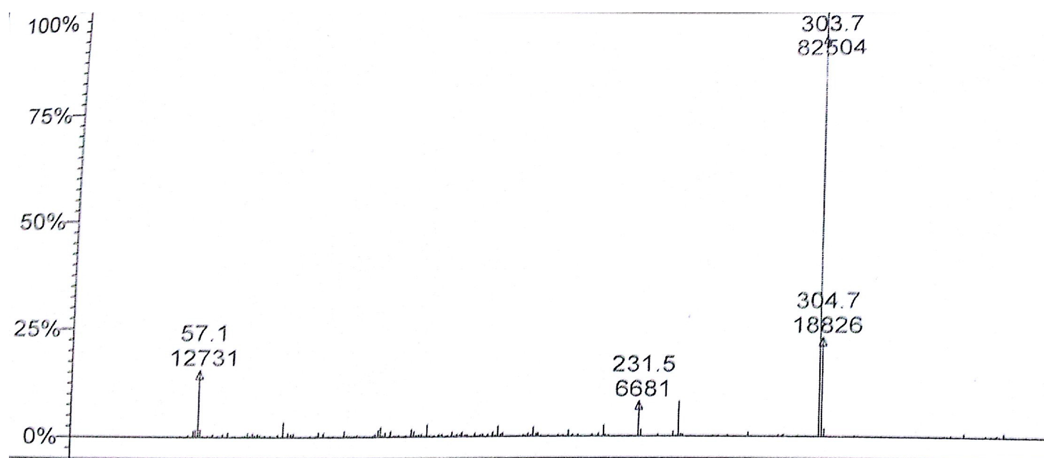

Figure-5d
GC-MS mass spectrum of AMR-7

The azo dyes are degraded by bacteria using their extracellular hydrolytic and oxidative enzymes[24]. Earlier endophytes with potential for bioremediation have been studied[25]. The pathway for biodegradation of Reactive Black-5 was attempted to propose as indicated in figure-6, showing various steps that might be involved in the biodegradation. However, very little is

known about the nature of biodegradation, the metabolites formed and the mechanism by which the enzymes act upon. It was proposed that initially primary asymmetric cleavage of azo bond of reactive black-5 resulted in the formation of three compounds: intermediate-1 with molecular weight 300 (m/z 303) figure-5d and intermediate-2 with molecular weight 347 and intermediate-3 with molecular weight 300 (m/z 303) figure-5d. The biotransformation of intermediate 1 resulted in the formation of a compound with mass peak at 198, followed by conversion to compound with molecular weight 106 and the end product as benzene with molecular weight 78(m/z 82.2) figure-5a whereas the biotransformation of intermediate 2 resulted in the formation of compound with molecular weight 245 (m/z 247.5) figure-5c which upon further action by enzymes formed compound with molecular weight 143 and a mass peak at 145 Figure-5b and the end product was naphthalene with molecular weight 128 and mass peak at 128.3 figure-5a while biotransformation of intermediate 3 resulted in the formation of a compound with mass peak at 198, followed by conversion to compound with molecular weight 106 and the end product as benzene with molecular weight 78(m/z 82.2) Figure-5a. On the other hand the culture AMR-7 showed transformation to compounds with comparatively higher molecular weights. The sequential reactions resulted in complete biodegradation of the dye Reactive Balck-5 and the formation of low molecular weight compound such as naphthalene and benzene as end products which are non-toxic. In the present study the metabolites obtained due to biodegradation of the selected azo dye were of much lower mass than those of the original azo dye compounds[26]. The GC-MS mass spectra of the AMR-1 showed biodegradation of the selected dye to benzene and naphthalene which are non-toxic.

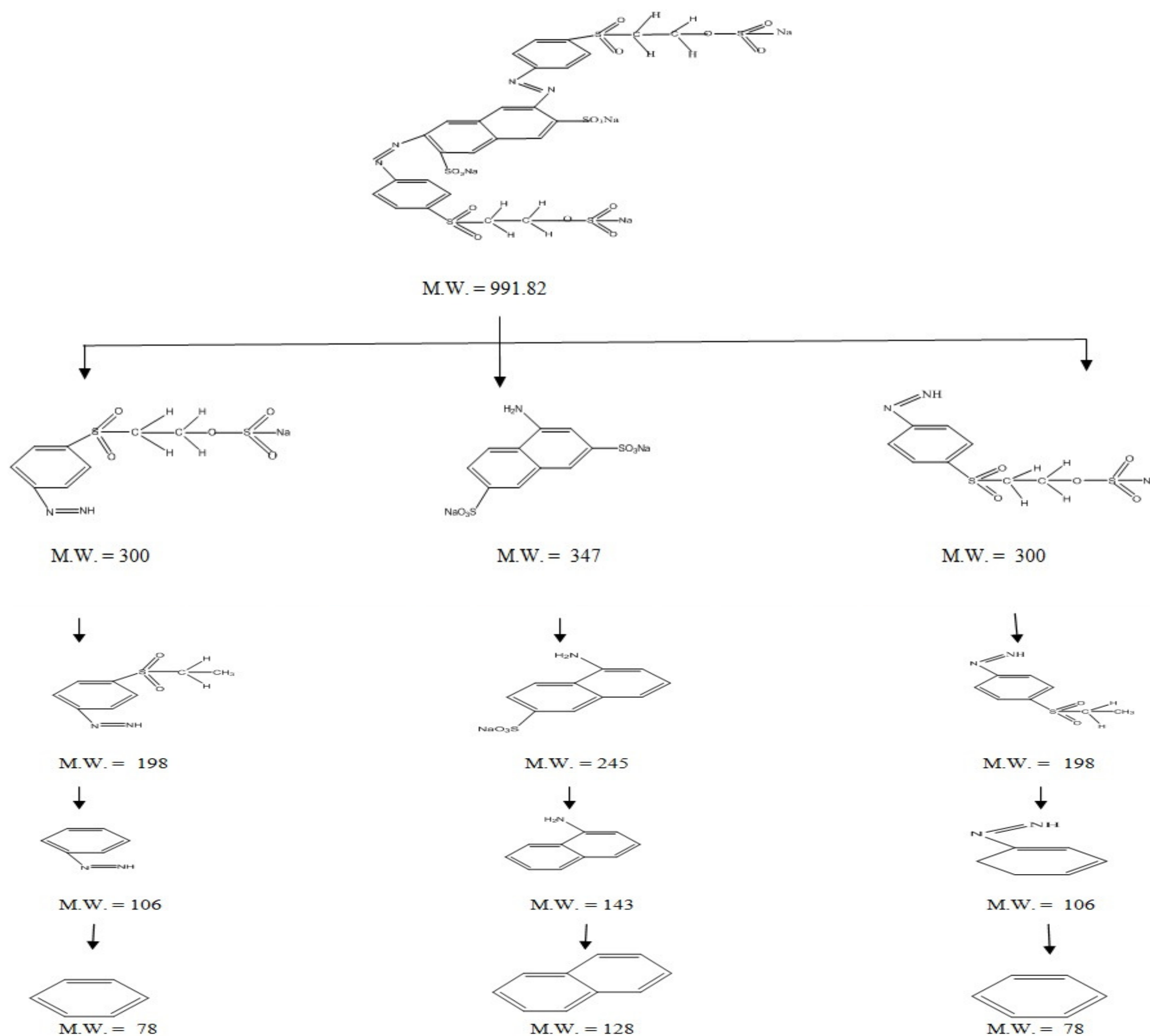

Figure- 6
Proposed biodegradation pathway of dye Reactive Black-5 by *Exiguobacterium profundum*

The biodegradation potential of *Exiguobacetrium profundum strain 4N* was confirmed by analytical studies. Earlier also *Exiguobacterium* had been reported for their tolerance and resistance to arsenic[27], benzene toluene and xylene[28], selenite[29] and chromate[30]. The bacterium might be harboring an elaborate array of diverse enzymes which could biodegrade variety of organic compounds. The consecutive action of oxidoreductive enzymes on the dye Reactive Black-5 resulted in the formation of smaller molecular weight intermediates and the formation of benzene and naphthalene as end products which are nontoxic in nature. The metabolites formed during biodegradation of reactive black-5 were the result of several reactions like: oxidation, reduction, desulfonation, demethylation etc. The most important step in the whole pathway was the breakdown of the azo bond. The biotransformation of azo dye by *Exiguobacetrium profundum strain 4N* into non-toxic form is very important in order to remove these pollutants from the environment.

Phytotoxicity study: The toxicity studies on Reactive Black-5 (untreated) and the extracted metabolites (treated) obtained after the biodegradation of the selected azo dye by the competent and efficient bacterial endophyte AMR-1, on plants *Triticum aestivum* (monocot) and *Phaseolus mungo* (dicot) showed that the extracted metabolites were non toxic and did not interfere with the germination and growth (radical and plumule length) of the plant seeds table-2 rather their effect on growth and germination was comparable with distilled water, however the dye Reactive Black-5 was observed to be considerably toxic on the growth and germination of the selected plant seeds.

Conclusion

A novel bacterial endophyte *Exiguobacterium profundum strain N4* isolated from plant *Amaranthus spinosus,* was found competent and efficient in decolorizing, transforming and degrading azo dye Reactive Black-5. The study provides a cost-effective, safe and eco-friendly solution for the removal of toxic textile azo dyes from the environment. Moreover, the plant harboring this competent and efficient bacterial endophyte could be employed for phytoremediation purpose so as to remove toxic azo dyes. The plant has the ability of not only tolerating the stress of toxic azo dyes but also tends to flourish in such a contaminated environment.

Acknowledgement

The authors are sincerely thankful to the Department of Botany, University of Rajasthan, Jaipur, for providing necessary facilities. The authors are also thankful to IARI for providing support and necessary facilities in conducting analytical studies.

References

1. Siciliano S.D., Fortin N., Mihoc A., Wisse G., Labelle S., Beaumier D., Ouellette D., Roy R., Whyte L. G., Banks M.K., Schwab P., Lee K. and Greer C.W., Selection of specific endophytic bacterial genotypes by plants in response to soil contamination, *Appl. Environ. Microbiol.*, **67**, 2469-2475 **(2001)**
2. Rosenblueth M. and Martinez Romero E., Review-Bacterial endophytes and their interactions with hosts, *Molecular Plant-Microbe Interaction*, **19(8)**, 827–837 **(2006)**
3. Van Aken B., Peres C., Doty S., Yoon J. and Schnoor J., *Methylobacterium populi* sp. nov., a novel aerobic, pink-pigmented, facultative methylotrophic, methane-utilising bacterium isolated from poplar trees (*Populus deltoids x nigra* DN34). *Evol. Microbiol*., **54**, 1191–1196 **(2004a)**
4. Van Aken B., Yoon J. and Schnoor J., Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine inside poplar tissues (*Populus deltoids x nigra* DN34), *Appl. Environ. Microbiol.*, **70**, 508–517 **(2004b)**
5. Germaine K.J., Liu X., Cabellos G.G., Hogan J.P., Ryan D. and Dowling D.N. Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid, *FEMS Microbiol. Ecol*., **57**, 302–310 **(2006)**
6. Tamura K., Dudley J., Nei M. and Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0, *Molecular Biology and Evolution*, **24**,1596-1599 **(2007)**
7. Saitou N. and Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees, *Molecular Biology and Evolution*, **4**, 406-425**(1987)**

Table- 2
Phytotoxicity study of dye Reactive Black-5 and its extracted metabolites

Parameters Studied	Triticum aestivum			Phaseolus mungo		
	Distilled Water	Black-5	Extracted metabolite	Distilled Water	Black-5	Extracted metabolite
Germination %	100	80	90	100	60	70
Root (cm)(±SD)	7.3+0.10	4.7+0.06	6.9+0.42	7.9+ 0.38	5.3+ 0.29	7.3+0.10
Shoot (cm)(±SD)	12.8+0.15	5.7+0.21	11.6+0.12	14.2+ 0.42	7.1+0.15	12.1+0.35

Values are the mean of experiment conducted in triplicate, SEM (±)

8. Sturz A. V., Christie B. R. and Nowak J., Bacterial endophytes: Potential role in developing sustainable systems of crop production, *Crit. Rev. Plant Sci*., **19**, 1-30 **(2000)**

9. Strobel G.A., Daisy B., Castillo U. and Harper J., Natural products from endophytic microorganisms, *J. Nat. Prod*., **67**, 257–268 **(2004)**

10. Pandey A., Singh P. and Iyengar L., Bacterial decolorization and degradation of azo dyes, *Int. Biodeterior. Biodegrad*., **59**, 73–84 **(2007)**

11. Zhou W. and Zimmermann W., Decolorization of industrial effluents containing reactive dyes by actinomycetes, *FEMS Microbiology Letters*, **107(2-3)**, 157–161 **(1993)**

12. Bheemaraddi Mallikarjun C., Patil Santosh and Shivannavar Channappa T., Gaddad, Subhash chandra M. Isolation and characterization of *Paracoccus* sp. GSM2 capable of degrading textile azo dye Reactive Violet 5, *Scientific World Journal*, doi: 10.1155/2014/410704 **(2014)**

13. Ambrósio S.T. and Campos-Takaki G.M., Decolorization of reactive azo dyes by *Cunninghamella elegans* UCP 542 under co-metabolic conditions, *Bioresour. Technol.,* **91**, 69-75 **(2004)**

14. Saratale R.G., Gandhi Soniya S., Purankar Madhavi V., Kurade Mayur B., Govindwar Sanjay P., Oh Sang Eun and Saratale G.D., Decolorization and detoxification of sulfonated azo dye C.I. Remazol Red and textile effluent by isolated *Lysinibacillus* sp. RGS, *Journal of Bioscience and Bioengineering*, **20(20)**, 1-10 **(2012)**

15. Chen K.C., Wu J.Y., Liou D.J. and Hwang S.C.J., Decolorization of the textile dyes by newly isolated bacterial strains, *J. Biotechnol*., **101(1)**, 57-68 **(2003)**

16. Dhanve R.S., Shabdalkar U.U. and Jadhav J.P., Biodegradation of diazo reactive dye Navy blue HE2R (Reactive blue 172) by an isolated *Exiguobacterium* sp. RD3, *Biotechnology and Bioprocess Engineering*, **13**, 53-60 **(2008)**

17. Moosvi S., Keharia H. and Madamwar D., Decolorization of textile dye Reactive Violet 5 by a newly isolated bacterial consortium RVM 11.1, *World J. Microbiol. Biotechnol*., **21**, 667–672 **(2005)**

18. Kalyani D.C., Patil P.S., Jadhav J.P. and Govindwar S.P., Biodegradation of reactive textile dye Red BLI by an isolated bacterium *Pseudomonas* sp. SUK1, *Bioresource Technology*, **99**, 4635–4641 **(2008)**

19. Rodrigues D.F., Goris J., Vishnivetskaya T., Gilichinsky D., Thomashow M.F. and Tiedje J.M., Characterization of *Exiguobacterium* isolates from the Siberian permafrost, Description of *Exiguobacterium sibiricum* sp. nov. *Extremophiles*, **10(4)**, 285-94 **(2006)**

20. Singh N.K., Raichand R., Kaur I., Kaur C., Pareek S. and Mayilraj S., *Exiguobacterium himgiriensis* sp. nov. a novel member of the genus *Exiguobacterium*, isolated from the Indian Himalayas, *Antonie Van Leeuwenhoek*, **103(4)**, 789-96 **(2013)**

21. Wang X., Cheng X., Sun D. and Qi H., Biodecolorization and partial mineralization of Reactive Black 5 by a strain of *Rhodopseudomonas palustris*, *J. Environ. Sci. (China)*, **20(10)**, 1218-25 **(2008)**

22. Hussain S., Maqbool Z., Ali S., Yasmeen T., Imran M., Mahmood F. and Abbas F., Biodecolorization of Reactive Black-5 by a metal and salt tolerant bacterial strain *Pseudomonas* sp. RA20 isolated from Paharang drain effluents in Pakistan, *Ecotoxicol. Environ. Saf.,* **98**, 331-8 **(2013)**

23. Tan L., Qu Y.Y., Zhou J.T., Li A. and Gou M., Identification and characteristics of a novel salt-tolerant *Exiguobacterium* sp. for azo dyes decolorization, *Appl. Biochem. Biotechnol*., **159(3)**, 728-738 **(2009)**

24. Wang H., Su J.Q., Zheng X.W., Tian Y., Xiong X.J. and Zheng T.L., Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by *Citrobacter* sp. CK3, *International Biodeterioration and Biodegradation,* **63**, 395-399 **(2009)**

25. Mannisto M.K., Tiirola M.A. and Puhakka J.A., Degradation of 2, 3, 4, 6-tetraclorophenol at low temperature and low dioxygen concentrations by phylogenetically different groundwater and bioreactor bacteria, *Biodegradation*, **12**, 291–301 **(2001)**

26. Shertate Rubina and Thorat Prakash, Biotransformation of a textile azo dye Mordant Orange 1 by *Halobacillus Trueperi* MO-22 in marine condition, *Int. J. of Pharm. Phytopharm Res.,* **3**, 268-276 **(2013)**

27. Belfiore C., Ordoñez O.F. and Farías M.E., Proteomic approach of adaptive response to arsenic stress in *Exiguobacterium* sp. S17, an extremophile strain isolated from a high-altitude Andean Lake stromatolite. *Extremophiles*, **17(3)**, 421-31 **(2013)**

28. Wang L., Qiao N., Sun F. and Shao Z., Isolation, gene detection and solvent tolerance of benzene, toluene and xylene degrading bacteria from near shore surface water and Pacific Ocean sediment, *Extremophiles*, **12(3)**, 335-42 **(2008)**

29. Ikram M. and Faisal M., Comparative assessment of selenite (SeIV) detoxification to elemental selenium (Se0) by *Bacillus* sp. *Biotechnol Lett*., **32(9)**,1255-9 **(2010)**

30. Alam M.Z. and Malik A., Chromate resistance, transport and bioreduction by *Exiguobacterium* sp. ZM-2 isolated from agricultural soil irrigated with tannery effluent, *J. Basic Microbiol*., **48(5)**, 416-20 **(2008)**